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Functional Studies of a Germ-Line Polymorphism at Codon 47 within the p53 Gene

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Summary

A rare germ-line polymorphism in codon 47 of the p53 gene replaces the wild-type proline (CCG) with a serine (TCG). Restriction analysis of 101 human samples revealed the frequency of the rare allele to be 0% (n = 69) in Caucasians and 4.7% (3/64, n = 32) among African-Americans. To investigate the consequence of this amino acid substitution, a cDNA construct (p53 mut47ser) containing the mutation was introduced into a lung adenocarcinoma cell line (Calu-6) that does not express p53. A growth suppression similar to that obtained after introduction of a wild-type p53 cDNA construct was observed, in contrast to the result obtained by introduction of p53 mut143ala. Furthermore, expression of neither p53 mut47ser nor wild-type p53 was tolerated by growing cells. In transient expression assays, both mut47ser and wild-type p53 activated the expression of a reporter gene linked to a p53 binding sequence (PG13-CAT) and inhibited the expression of the luciferase gene under the control of the Rous sarcoma virus promoter (RSVluc). In the same assay, mut143ala did not activate the expression of PG13-CAT and produced only a slight inhibitory effect on RSVluc. These findings indicate that the p53 variant with a serine at codon 47 should be considered as a rare germ-line polymorphism that does not alter the growth-suppression activity of p53.

Introduction

p53 protein plays an important role in the regulation of cell growth. Apparently its function is dependent upon its structure, because several mutant forms that induce new epitopes (Finlay et al. 1988; Gannon et al. 1990) can immortalize rodent cells in culture (Jenkins et al. 1984), cooperate with the ras oncogene to transform rodent cells in culture (Eliyahu et al. 1984; Jenkins et al. 1984; Parada et al. 1984), and cooperate with SV40 T in the neoplastic transformation of human bronchial epithelial cells (Gerwin et al. 1992). Information on the tertiary structure of the p53 protein is not yet available, since it has not been crystallized; however, some inferences can be derived from analysis of cDNA and protein sequences in different species (Zakut-Houri et al. 1985).

Several polymorphisms have been described for the p53 gene (Harris et al. 1986; Matlashewski et al. 1987; Ahuja et al. 1990; Carbone et al. 1991; Toguchida et al. 1992), but few have been characterized. The codon 72 (arg→pro) polymorphism alters the electrophoretic mobility of the protein (Harris et al. 1986). The distribution of the codon 72 polymorphism was found to differ significantly between Caucasians and African-Americans, and there was a tendency for the proline allele to be overrepresented in patients with lung adenocarcinoma (Weston et al. 1992). Very recently, the analysis of germ-line mutations of the p53 tumor-suppressor gene in patients with the Li-Fraumeni syndrome revealed that one of seven missense mutations (codon 181, arg→his) retained some in vitro activities of wild-type p53 (wt p53) (Frebourg et al. 1992*a*, 1992*b*). In the present study, using a similar functional approach, we have characterized a new and rare p53 polymorphism at codon 47 (CCG^{pro}→TCG^{ser}) by expressing a protein with this mutation in a human lung adenocarcinoma

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cell line (Calu-6) in which p53 is not usually detected (Caamano et al. 1991; Lehman et al. 1991). By comparison with experiments introducing either wt p53 or p53 mut143ala, we have shown that the C→T substitution in codon 47 does not appear to alter the wild-type characteristics of the protein, despite the fact that 1 of the 23 proline residues in the transactivation domain is replaced by a serine residue.

Material and Methods

Polymorphism Detection

A PCR/restriction digest–genotyping test was developed on the basis of the abolition of an *Nci*I restriction site by the C→T transition at codon 47. Genomic DNA was extracted from non-neoplastic human lung tissues of 101 subjects obtained at autopsy from University of Maryland Hospital at Baltimore. DNA samples (500 ng) were suspended in buffer (15 mM Tris pH 8.0, 50 mM KCl, 2 mM $MgCl_2$, 0.3 mM dNTP, containing TGAGGACCTGGTCCTCTGAC and AGAGGAATCCCAAAGTTCCA [0.4 μM] as 5′ and 3′ primers, respectively) and amplified for 35 cycles (94°C for 40 s; 60°C for 1 min; and 78°C for 30 s). The resulting PCR products (420 bp) were digested to completion with *Nci*I and analyzed by agarose gel electrophoresis.

Cell Line

Calu-6 is a lung adenocarcinoma cell line that contains a termination codon at position 196 of p53 and does not express detectable levels of p53 mRNA and protein (Caamano et al. 1991; Lehman et al. 1991). Furthermore, a karyotype analysis of the cells utilized in this study (Children's Hospital of Michigan, Detroit) revealed complete loss of the chromosome 17 pair, a pattern frequently observed in the Calu-6 cell line. Cells were grown in HUT medium (RPMI 1640, 20 mM Hepes, 10% fetal bovine serum [FBS]) supplemented with antibiotics.

Recombinant Plasmids

pC53-SN (i.e., wt p53), pC53-SCX3 (i.e., p53 mut143ala), and pCMV-NEO-BAMHI (cmvneo) control vector were provided by Dr. B. Vogelstein (Johns Hopkins University Medical Center, Baltimore). In these constructs the p53 gene is controlled by the CMV promoter, and the neo gene (Tn5) is controlled by the thymidine kinase promoter. Cmvneo47 was constructed by site-directed mutagenesis. In brief, the wt p53 cDNA fragment was inserted into the *Bam*HI site of the pSelect vector (Promega), containing a nonfunctional Amp R gene. A 20-bp primer harboring the desired p53 mutation (5′ATATCGTCCGAGGACAGCAT) and a second primer which would restore Amp R in the plasmid were annealed after single-stranded DNA production. Plasmids were selected on the basis of restored Amp R, as described in the Promega instruction manual. The reporter plasmids PG13-CAT and MG15-CAT were also gifts of Dr. B. Vogelstein. PG13-CAT contains 13 copies of nucleotides 103–134 from fragment A which correspond to the p53 binding site located in the upstream region of the ribosomal gene cluster (Kern et al. 1991). MG-15 contains a mutated p53 binding site sequence which is not bound by either wt p53 or mutant p53 proteins. Rous sarcoma virus promoter (RSVluc) was a gift of Dr. B. Paterson and contains the luciferase gene under the control of Rous sarcoma virus LTR (de Wet et al. 1987).

Transfections

All transfection experiments were performed with Calu-6 cells (between passages 52 and 60) at 80% confluence in 10-cm Petri dishes. For transfection of the p53 and CMV control constructs, 10 μg of plasmid DNA, together with 25 μg of Lipofectin (Bethesda Research Laboratories, Gaithersburg, MD), were mixed together and then added to HUT medium without FBS (5 ml/dish), according to manufacturer's instructions. After 5 h, the medium was replaced by HUT medium with 10% FBS, and selection with 75 μg G418/ml (geneticin®; Gibco BRL) was begun 48 h after transfection. After 14 d of selection, a mass culture (experiment 1) and clonal subpopulations (experiment 2) were established and expanded.

For transient expression assays, the reporter plasmids PG13-CAT (0.75 μg) and MG15-CAT (0.75 μg) and RSVluc (1.5 μg) were cotransfected with p53 expression vectors (2.5 μg) by using the lipofection procedure and Calu-6 cells, under conditions that were the same as those described above. The CAT and luciferase activities were analyzed after 24 h, using Promega reagents and protocols (Promega, Madison).

Southern Blot Analysis

DNA was extracted as follows: Cells were washed once with PBS and were resuspended in lysis buffer (200 mM NaCl, 50 mM Tris pH 7.4, 0.4% SDS, 5 mM EDTA). Proteinase K was added to a final concentration of 0.1 mg/ml. Digestion (at 60°C for 2 h) was followed by phenol:chloroform:isoamylalcohol (25:24:1) extraction, and DNA was precipitated by the addition of 2.5 vol of ice-cold ethanol. *Bam*HI-digested

Table 1

Ethnic Distribution of the Polymorphism at Codon 47 of p53

	No. of Subjects	No. with Allele Combinations[a]		
		pro/pro	pro/ser	ser/ser
African-American	32	29	3[b]	0
Caucasian American	69	69	0	0

[a] pro/pro = proline homozygote; pro/ser = heterozygote; and ser/ser = serine homozygote. The serine allele lacks the *Nci*I restriction site, and the proline allele contains the *Nci*I restriction site.

[b] The distribution of the serine allele is significantly different ($P < .05$; Fisher's exact test) between African-Americans and Caucasians.

samples were analyzed in a Southern blot hybridization (16 h at 65°C) to the *Xba*I fragment of p53 cDNA (1.8 kb) that had been labeled by random priming. Blots were washed twice at low stringency (2 × SSC, 25°C), then at increasingly stringent conditions (65°C; 2 × SSC/1% SDS, 1 × SSC/1% SDS, 0.5 × SSC/1% SDS, and 0.5 × SSC/1% SDS).

Northern Blot Analysis

RNA was prepared from subconfluent cultures by acid-guanidinium extraction (Chomczynski and Sacchi 1987). RNA samples (20 μg) were hybridized (16 h at 42°C) to the 1.2-kb p53 coding region or the 1.5-kb rat glyceraldehyde-phosphate dehydrogenase (GAPDH) labeled by random priming. Hybridized filters were washed at 65°C by following the same protocol as that for Southern blotting.

Immunocytochemical Analysis

Cells were seeded onto glass multiwell chamber slides (LAB-TEK, Nunc, Naperville, IL), incubated overnight (37°C in 3.5% CO_2 at 95% humidity), and fixed in acetone (−20°C for 10 min). Endogenous peroxidase activity was quenched (20 min at room temperature with a 0.3% hydrogen peroxide solution in PBS). Antigenic cross-reactivity was blocked with normal horse serum (1:50 dilution). Saturating concentrations of murine monoclonal primary antibodies were incubated overnight at 4°C and were subsequently detected by a biotinylated secondary antibody and an avidin-biotin peroxidase system, according to the manufacturer's protocol (Vecta-Stain Elite Kit; Vector Laboratories, Burlingame, CA). The chromogen was diaminobenzidine (final concentration 0.05 mg/ml) with nickel chloride (final concentration 0.03%); there was no counterstain. A proliferation marker, Ki-67, demonstrated the viability of the cell cultures (working dilution 1:1,000, M722; Dakopatts, Glostrup, Denmark). A monoclonal antibody to SV 40 large T antigen served as an isotype-matched negative control (working dilution 1:500, PAB 416, AB-2; Oncogene Science, Manhasset, NY).

p53 protein accumulation was demonstrated by concordant staining with multiple monoclonal antibodies, including PAB 1801 (working dilution 1:1,000, AB-2; Oncogene Science, Manhasset, NY), PAB 122 (working dilution 1:300, 14091A; Pharmingen, San Diego), DO-1 (working dilution 1:1,000, provided by Dr. David P. Lane, University of Dundee, U.K.), and BP-53-12 (working dilution 1:25, MA195-5C; Biogenex, San Ramon, CA). Intense extranucleolar, nuclear staining was the criterion for a positive reaction.

Amplification of the Neo Gene

The presence of the neo gene from the recombinant plasmid was determined by PCR. DNA (200–500 ng) isolated from the cells was suspended in a reaction buffer (67 mM Tris-HCl pH 8.8, 6.7 mM $MgCl_2$, 16.6 mM $(NH_4)_2SO_4$, 6.8 μM EDTA, 10 mM 2-mercaptoethanol, 1 mM dNTP) and was subjected to 35 cycles of denaturation (40 s at 94°C), annealing (1 min at 60°C), and elongation (30 s at 78°C) in the presence of *Taq* polymerase (1 U; Cetus) and neo-specific 5′ and 3′ primers ACGTGAATTCGATGGATTGCACGCAGGTTC and CGATGGATCCGCAGGAGCAAGGTGAGAGATGA (1 μM each), respectively. Amplification produced a 330-bp fragment.

Growth Rate

Growth rates were determined by plating cells in triplicate dishes at 10^3 cells/60-mm dish and staining 3 dishes/d at 24-h intervals for 8 d. Cells were rinsed in PBS, fixed for 10 min (4% formaldehyde), and stained

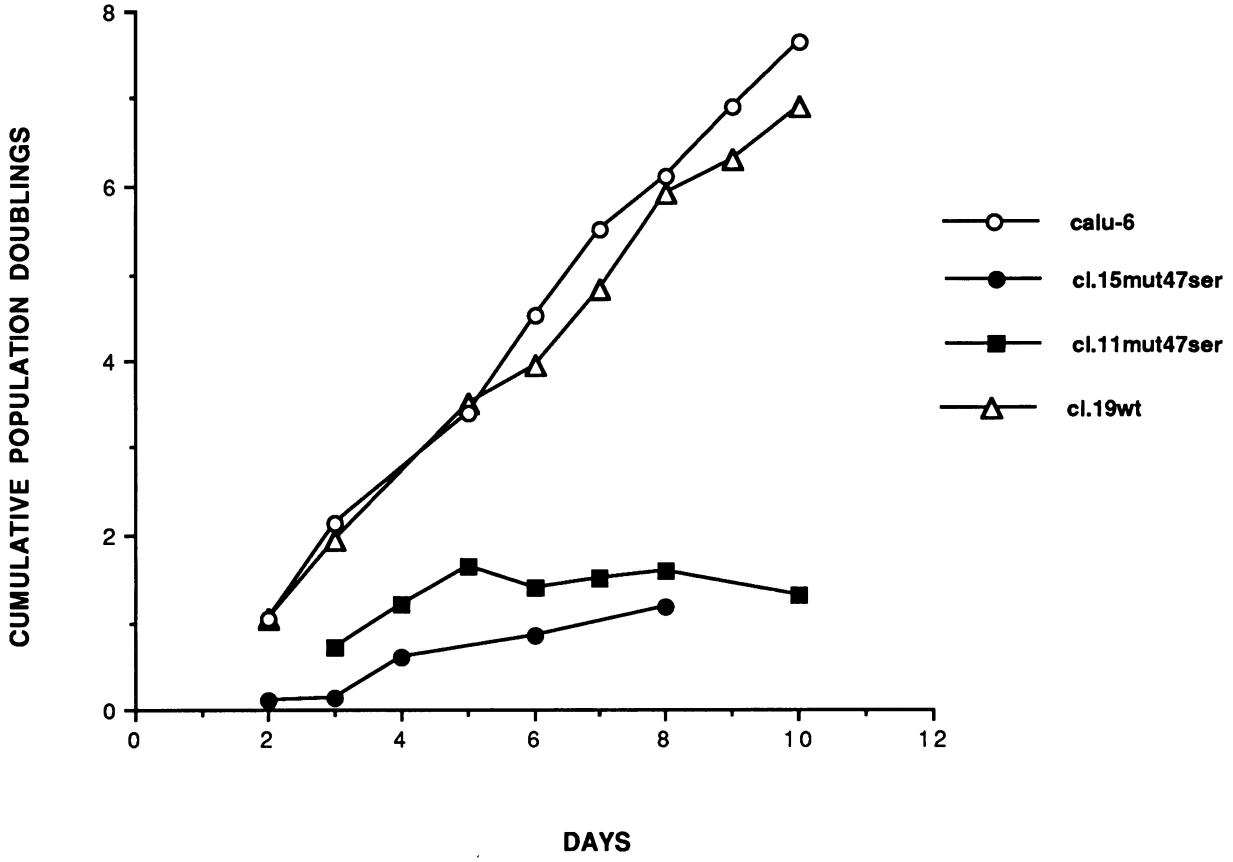

Figure 1 Growth rate of Calu-6 and cell lines derived from transfection of mut47ser (cl. 11 and 15) or wt p53 (cl. 19). Cells were plated in triplicate at a density of 35 cells/cm^2, and the number of cells per colony was counted in 60 colonies per line on several days between days 2 and 10.

for 5 min (0.25% crystal violet). The number of cells per colony was determined using the Bioquant System IV Image Analysis (R & M Biometrics, Nashville). The number of cells was determined in 20 colonies/dish, and population doublings (pd) are expressed as $\log_2$(cells per colony).

Results

Distribution of p53 Alleles

The PCR analysis with restriction enzyme digestion was performed on genomic DNA samples from 32 African-Americans and 69 Caucasians (table 1). The overall serine allele frequency was 1.5%; however, this allele was found only in African-Americans, and, in that group, the frequency was 4.7% (3/64 alleles). According to the Hardy-Weinberg equilibrium, the frequency of the ser/ser allele in the African-American population would be 1/500; thus, a homozygous mutant would not be expected to be detected in the studied population.

Colony Formation Efficiency and Clonal Growth Rates

After introduction of wt-, mut47ser-, and mut143ala-p53 constructs into Calu-6, geneticin-resistant colonies were counted 3 wk later. Calu-6 cells transfected with pC53-SN3 (wt p53) and cmvneo47 (mut47ser) formed 35 and 8 colonies, respectively, while 108 and 167 colonies were obtained in cells transfected with pC53-SCX3 (mut143ala) and the cmvneo control vector, respectively. These results indicate that both the wt p53 and the mut47ser inhibited the clonal growth of Calu-6.

To characterize the geneticin-resistant colonies, some were expanded into cell lines. During this process we observed that the cell lines derived from cmvneo47 transfection (clone [Cl.] 10, 11, 14, and 15) had a lower growth rate when compared with the clones derived

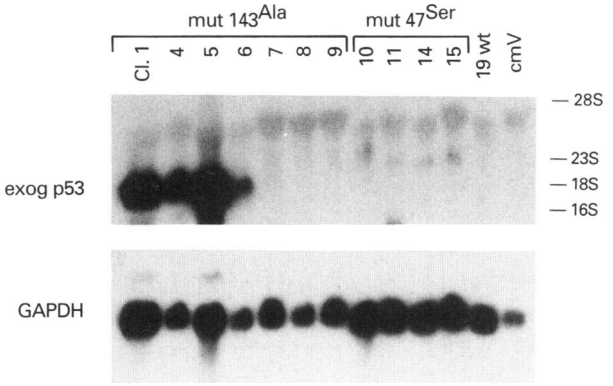

Figure 2 Northern analysis of RNA extracted from Calu-6 cells transfected with mut143ala p53 (Cl. 1, 4, 5, 6, 7, 8, and 9), mut47ser (Cl. 10, 11, 14, and 15), wt p53 (Cl. 19), or control vector (cmV). Exogenous p53 expression was determined by hybridization with the p53 coding region (1.2 kb) and was compared with the expression of endogenous GAPDH.

from transfection of wt p53 (Cl. 19 and 20) or mut143ala (Cl. 1, 4, 5, 6, 7, 8, and 9). Generally mut47ser clones reached 28 pd 3 wk later than clones transfected with wt p53 or mut143ala. When mut47ser clones (Cl. 11mut47 and Cl. 15mut47) were plated after 26 pd at a low clonal density, a dramatic growth suppression was observed in comparison with Cl. 19wt (fig. 1).

p53 Expression and Activity

To determine whether the observed growth pattern correlated with p53 expression, RNA harvested after 28 pd was analyzed with a p53 probe. Four (Cl. 1, 4, 5, and 6) of seven clones derived from transfection with mut143ala were found to express the exogenous message (fig. 2). In contrast, none of the clones derived from wt p53 or mut47ser p53 expressed the exogenous gene (fig. 2). The same result was observed in the mass cultures derived from Calu-6–transfected cells in experiment 1 (data not shown). To buttress this correlation with mRNA expression, a subset of clones was examined for p53 protein accumulation (table 2). The untransfected Calu-6 culture had been previously shown to lack detectable levels of p53 protein (Lehman et al. 1991). The examined clones included one wild-type, one mut47ser, and two mut143ala clones. One of the mut143ala clones (Cl. 1) expressed p53 mRNA, and this was the only clone to accumulate high levels of p53 protein. The remaining clones were negative for p53 mRNA, and all contained less than 3.4% positive cells. These results complement the northern analysis results, which would not be expected to detect mRNA from such a small subpopulation.

To further investigate these results, DNA samples from the cell cultures were examined for the exogenous p53 cDNA sequence. All the p53 clones, which expressed p53 mRNA, plus clone 8, which did not ex-

Table 2

p53 mRNA and Protein in Calu-6 Cultures Transfected with Wild-Type and Mutant p53 Constructs

Transfected p53 Construct	Culture Type	mRNA[a]	Protein Accumulation[b]	
			% of Positive Nuclei	No. of Positive Cells/ Total
None	Mass culture	–[c]	0	N/A
Wild type	Clone 19	–	3.4	15/435
Mut47ser	Clone 11	–	<.1	N/A
Mut143ala	Clone 1	+	64.5	240/372
Mut143ala	Clone 7	–	1.8	8/435

[a] p53 mRNA detected by northern blot; a minus sign indicates absence, and a plus sign indicates presence.

[b] Quantitation of p53 protein accumulation was performed by manual counts of sequential cells. Three staining categories were identified: cells with intense nuclear, extranucleolar staining are positive; cells with negative nuclei are negative; and cells with faintly stained nuclei with or without cytoplasmic staining are intermediate (+/–); fragmented and mitotic cells were not counted. The ratios (e.g., 15/435) are no. of positive cells/no. of positive plus negative cells (i.e., the +/– negative nuclei are excluded). All ratios are composed of two independent experiments. N/A = not applicable. The percentage of positive nuclei was calculated excluding the +/– nuclei.

[c] Caamano et al. 1991; Lehman et al. 1991.

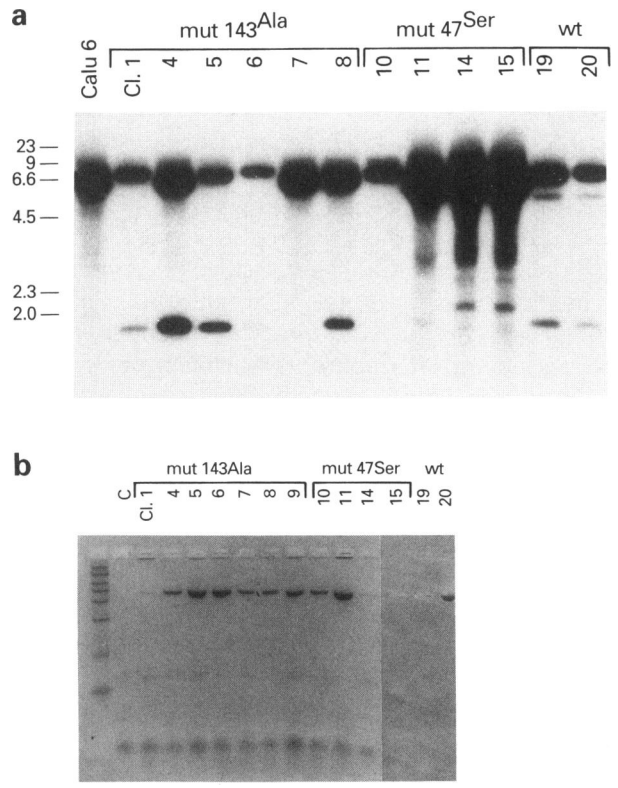

Figure 3 *a*, Southern analysis of DNA extracted from Calu-6 cells transfected with mut143ala (Cl. 1, 4, 5, 6, 7, and 8), mut47ser (Cl. 10, 11, 14, and 15), or wt p53 (Cl. 19 and 20). The presence of the exogenous p53 was determined in *Bam*HI-digested genomic DNA. Intact exogenous p53 has a size of 1.8 kb. *b*, Detection of the neo gene by PCR analysis in DNA extracted from Calu-6 cells transfected with mut143ala (Cl. 1, 4, 5, 6, 7, and 8), mut47ser (Cl. 10, 11, 14, and 15), or wt p53 (Cl. 19 and 20). Aliquots of the PCR products were electrophoresed on 4% NuSieve-agarose gels and stained with ethidium bromide. The first lane contains Gelmarker I (molecular weight 1,000, 700, 500, 400, 300, 200, 100, and 50 bp).

press p53 mRNA, contained an intact copy of the exogenous p53 construct (fig. 3*a*). However in clone 7 (mut143ala) and in all four clones from mut47ser, the exogenous p53 sequences were deleted or rearranged (fig. 3*a*). In contrast, the exogenous p53 gene was also intact in both wild-type–derived clones; these two clones also showed an unidentified band of high-molecular-weight DNA hybridizing with the p53 probe. All of the clones contained the neo gene (fig. 3*b*). Thus, both mut47ser and wt p53 were inactivated, although by different mechanisms.

To evaluate the activities of the different p53 vectors as transcription modulators, transient expression assays were performed. In agreement with the stable expression results, mut47ser showed a transactivation of the PG13-CAT construct similar to that of wt p53, whereas mut143ala was totally ineffective (table 3). Luciferase activity was analyzed with the initial purpose of correcting for transfection efficiency. In the presence of wt p53 or mut47ser p53, only 30% of the luciferase activity was observed, in comparison with the result obtained with the control vector (table 3). The luciferase activity was also slightly decreased in the presence of mut143ala.

Discussion

In this study we report that a mutation at codon 47 of p53 ($CCG^{pro} \rightarrow TCG^{ser}$), originally described in bronchial epithelial cells immortalized by the SV40 large T antigen and the donor's cells (Gerwin et al. 1992), is actually a rare p53 germ-line polymorphism that does not appear to alter the function of the protein. The allelic frequency of mut47ser p53 is 0% in the Caucasian population and 4.7% in the African-American population, which is less than the codon 72 arg/pro polymorphism that occurs in 35% of the population (de la Calle-Martin et al. 1990; Weston et al. 1992). Inherited forms of p53 mutations have been described in families

Table 3

Transcriptional Activity of Exogenous p53 Proteins Detected in Transient Expression Assays

Reporter Plasmid and Expression Plasmid	%[a]
PG13-CAT:	
cmvneo	0
wt p53	7.6 ± 3.1
mut47ser	9.2 ± 6.2
mut143ala	0
MG15-CAT:	
wt p53	0
RSVluc:	
cmvneo	100
wt p53	30.8 ± 14.7
mut47ser	24.8 ± 9.5
mut143ala	48.9–89.2[b]

[a] PG13-CAT (0.75 μg) or MG15-CAT (0.75 μg) and RSVluc (1.5 μg) were cotransfected with p53 expression vectors (2.5 μg) into Calu-6 cells. The CAT and luciferase activities of transfected cells were analyzed 24 h after the transfection. The CAT activity is expressed as a percent of ^{14}C-chloramphenicol conversion. The luciferase activity is expressed relative to that obtained with RSVluc and cmvneo (i.e., 100%). Values are expressed as mean ± SD for $n = 4$.

[b] Range obtained in three independent experiments.

with Li-Fraumeni syndrome (Malkin et al. 1990), in patients with two independently arising neoplasms (Malkin et al. 1992), and in patients with sarcomas (Toguchida et al. 1992), but not every germ-line mutation was associated with tumor development in a cancer-prone family (Frebourg et al. 1992*a*).

Functional analysis of the p53 with a serine at codon 47 was performed by introduction of a cDNA containing this polymorphism (mut47ser) into Calu-6 cells, a lung adenocarcinoma cell line that does not express p53 (Caamano et al. 1991; Lehman et al. 1991). The results indicate that mut47ser protein maintains similar growth-suppression capacity and transcription-transactivation activity as that of the wt p53 construct. Calu-6 cells could not tolerate the expression of either mut47ser or wt p53 during their growth. This result is similar to that obtained by introduction of wt p53 in NCI-H358 lung bronchoalveolar carcinoma, which carries a homozygous deletion at the p53 locus (Takahashi et al. 1992).

Four of seven lines derived from transfection with the construct containing the mut143ala were found to express the exogenous p53. This is consistent with results expected in human cells transfected with a vector containing two independent transcription units (Baker et al. 1990 and references therein). In some of the clones the expression of integrated p53 gene was restricted; this had already been observed in the K562 CML cell line and in SKOV-3 ovarian adenocarcinoma (Johnson et al. 1991), and the mechanism remains obscure.

The transactivation of PG13-CAT, which contains multiple copies of the p53 binding sequence, by p53 mut47ser demonstrated that this mutant retained wild-type activity (Kern et al. 1991; Frebourg et al. 1992*a*, 1992*b*) in this assay. A similar result was observed with the codon 181 his mutant p53 transfected into Saos-2 cells (Frebourg et al. 1992*a*). This is in contrast with mut143ala, which was completely unable to bind the PG sequence. Furthermore wt and mut47ser inhibited luciferase expression to the same extent (70%); this reporter expression is under the control of the Rous sarcoma virus LTR promoter which was recently shown to be down regulated by wt p53 in Hela cells (Subler et al. 1992). This is consistent with the inhibitory effect of wt p53 on several cellular promoters (Ginsberg et al. 1991; Santhanam et al. 1991; Agoff et al. 1993). Therefore, the p53 gene is shown to both transactivate and inhibit gene expression in the same experiment and highlights the difficulty in establishing an appropriate control for the evaluation of transfection efficiency. Nevertheless, the reproducibility of the results attests to the reliability of the data.

Several rare polymorphisms in the N-terminus of p53 (codons 11, 21, 31, and 49) have been recently described (Ahuja et al. 1990; Toguchida et al. 1992). The phenotypic consequences of these variants are not known.

In this study we show that replacing one of the proline residues in the "proline stretch" by a serine has no demonstrable effect on the ability of p53 to suppress growth and control transcription. Even though we cannot exclude that this polymorphism alters unexamined properties, mut47ser functioned as wt p53 in our model system.

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References

Agoff SN, Hou J, Linzer DI, Wu B (1993) Regulation of the human hsp70 promoter by p53. Science 259:84–87

Ahuja HG, Testa MP, Cline MJ (1990) Variation in the protein coding region of the human p53 gene. Oncogene 5:1409–1410

Baker SJ, Markowitz S, Fearon ER, Willson JK, Vogelstein B (1990) Suppression of human colorectal carcinoma cell growth by wild-type p53. Science 249:912–915

Caamano J, Ruggeri B, Momiki S, Sickler A, Zhang SY, Klein-Szanto AJ (1991) Detection of p53 in primary lung tumors and nonsmall cell lung carcinoma cell lines. Am J Pathol 139:839–845

Carbone D, Chiba I, Mitsudomi T (1991) Polymorphism at codon 213 within the p53 gene. Oncogene 6:1691–1692

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159

de la Calle-Martin O, Fabregat V, Romero M, Soler J, Vives J, Yague J (1990) AccII polymorphism of the p53 gene. Nucleic Acids Res 18:4963

de Wet JR, Wood KV, DeLuca M, Helinski DR, Subramani S (1987) Firefly luciferase gene: structure and expression in mammalian cells. Mol Cell Biol 7:725–737

Eliyahu D, Raz A, Gruss P, Givol D, Oren M (1984) Participa-

tion of p53 cellular tumour antigen in transformation of normal embryonic cells. Nature 312:646–649

Finlay CA, Hinds PW, Tan TH, Eliyahu D, Oren M, Levine AJ (1988) Activating mutations for transformation by p53 produce a gene product that forms an hsc70-p53 complex with an altered half-life. Mol Cell Biol 8:531–539

Frebourg T, Barbier N, Kassel J, Ng YS, Romero P, Friend SH (1992*a*) A functional screen for germ line p53 mutations based on transcriptional activation. Cancer Res 52:6976–6978

Frebourg T, Kassel J, Lam KT, Gryka MA, Barbier-Piraux N, Andersen TI, Borresen AL, et al (1992*b*) Germline mutations of the p53 tumor suppressor gene in patients with high risk for cancer inactivate the p53 protein. Proc Natl Acad Sci USA 89:6413–6417

Gannon JV, Greaves R, Iggo R, Lane DP (1990) Activating mutations in p53 produce a common conformational effect: a monoclonal antibody specific for the mutant form. EMBO J 9:1595–1602

Gerwin BI, Spillare E, Forrester K, Lehman TA, Kispert J, Welsh JA, Pfeifer AMA, et al (1992) Mutant p53 can induce tumorigenic conversion of human bronchial epithelial cells and reduce their responsiveness to a negative growth factor, transforming growth factor type B1. Proc Natl Acad Sci USA 89:2759–2763

Ginsberg D, Mechta F, Yaniv M, Oren M (1991) Wild-type p53 can down-modulate the activity of various promoters. Proc Natl Acad Sci USA 88:9979–9983

Harris N, Brill E, Shohat O, Prokocimer M, Wolf D, Arai N, Rotter V (1986) Molecular basis for heterogeneity of the human p53 protein. Mol Cell Biol 6:4650–4656

Jenkins JR, Rudge K, Currie GA (1984) Cellular immortalization by a cDNA clone encoding the transformation-associated phosphoprotein p53. Nature 312:651–654

Johnson P, Gray D, Mowat M, Benchimol S (1991) Expression of wild-type p53 is not compatible with continued growth of p53-negative tumor cells. Mol Cell Biol 11:1–11

Kern SE, Kinzler KW, Bruskin A, Jarosz D, Friedman P, Prives C, Vogelstein B (1991) Identification of p53 as a sequence-specific DNA-binding protein. Science 252:1708–1711

Lehman TA, Bennett WP, Metcalf RA, Reddel R, Welsh JA, Ecker J, Modali RV, et al (1991) p53 mutations, ras mutations and p53-heat shock 70 protein complexes in human lung cell lines. Cancer Res 51:4090–4096

Malkin D, Jolly KW, Piraux NB, Look AT, Friend SH, Gebhardt MC, Andersen TI, et al (1992) Germline mutations of the p53 tumor suppressor gene in children and young adults with second malignant neoplasms. N Engl J Med 326:1309–1315

Malkin D, Li FP, Strong LC, Fraumeni JF, Nelson CE, Kim DH, Kassel J, et al (1990) Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 250:1233–1238

Matlashewski GJ, Tuck S, Pim D, Lamb P, Schneider J, Crawford LV (1987) Primary structure polymorphism at amino acid residue 72 of human p53. Mol Cell Biol 7:961–963

Parada LF, Land H, Weinberg RA, Wolf D, Rotter V (1984) Cooperation between gene encoding p53 tumour antigen and ras in cellular transformation. Nature 312:649–651

Santhanam U, Ray A, Sehgal PB (1991) Repression of the interleukin 6 gene promoter by p53 and the retinoblastoma susceptibility gene product. Proc Natl Acad Sci USA 88:7605–7609

Subler MA, Martin DW, Deb S (1992) Inhibition of viral and cellular promoters by human wild-type p53. J Virol 66:4757–4762

Takahashi T, Carbone D, Nau MM, Hida T, Linnoila I, Ueda R, Minna JD (1992) Wild-type but not mutant p53 suppresses the growth of human lung cancer cells bearing multiple genetic lesions. Cancer Res 52:2340–2343

Toguchida J, Yamaguchi T, Dayton SH, Beauchamp RL, Herrera GE, Ishizaki K, Yamamuro T, et al (1992) Prevalence and spectrum of germline mutations of the p53 gene among patients with sarcoma. N Engl J Med 326:1301–1308

Weston A, Perrin LS, Forrester K, Hoover RN, Trump BF, Harris CC, Caporaso NE (1992) Allelic frequency of a p53 polymorphism in human lung cancer. Cancer Epidemiol Biomarkers Prevention 1:481–483

Zakut-Houri R, Bienz-Tadmor B, Givol D, Oren M (1985) Human p53 cellular tumor antigen: cDNA sequence and expression in COS cells. EMBO J 4:1251–1255